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de Boer, P A ; Crossley, R E ; Rothfield, L I

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DOI: https://doi.org/10.1128/jb.170.5.2106-2112.1988

JOURNAL OF BACTERIOLOGY, May 1988, p. 2106–2112
0021-9193/88/052106-07$02.00/0

Vol. 170, No. 5

Isolation and Properties of *minB*, a Complex Genetic Locus Involved in Correct Placement of the Division Site in *Escherichia coli*

PIET A. J. DE BOER,* ROBIN E. CROSSLEY, AND LAWRENCE I. ROTHFIELD

Department of Microbiology, University of Connecticut Health Center, Farmington, Connecticut 06032

Received 3 December 1987/Accepted 1 February 1988

Mutation of the *Escherichia coli* minicell locus (*minB*) results in aberrant placement of the division septum. In this paper we report the isolation and characterization of the *minB* locus. Replacement of the chromosomal *minB*$^+$ allele by cloned *minB* sequences containing transposon insertions resulted in the minicell phenotype, indicating that *minB*$^+$ function is required to maintain the normal division pattern. Paradoxically, overexpression of the locus also resulted in the minicell phenotype. The locus codes for several peptides whose expression is coordinately affected by transposon mutations that also eliminate *minB*$^+$ function. A subset of the *minB* peptides is sufficient to prevent minicell formation in *minB1* mutants or to induce minicell formation when overproduced in wild-type strains, implicating these peptides in the normal process of localization of the division site. The results indicate that *minB* is a complex locus whose expression must be maintained within certain limits to maintain the normal pattern of localization of the division septum.

In most cells that divide by binary fission, such as *Escherichia coli*, cell division occurs with high fidelity at the midpoint of the cell (20). The mechanisms used by the cell to identify the proper site and to localize the cell division machinery at this site are unknown. We are approaching this morphogenetic problem by studying bacterial mutants that are characterized by abnormalities in placement of the division septum.

The best-known class of bacterial mutants with defects in placement of the division site are the minicell mutants that have been described in both gram-positive and gram-negative species (9). Minicell mutants are characterized by the frequent occurrence of aberrant septation events in which the septum is placed near the cell pole, leading to the appearance of small spherical chromosomeless cells (minicells) together with filamentous cells of variable lengths. Minicell-producing strains have been used extensively as tools to identify plasmid-encoded polypeptides (22) and to study the topological distribution of cell components (9). Relatively little attention, however, has been given to the molecular defects responsible for the aberrant localization of the division site.

It was thought originally that mutations in two genetic loci (*minA* and *minB*) were required to bring about the minicell phenotype in *E. coli* (9). Recently, however, it was shown that mutation of a single locus (*minB*) is sufficient to induce the complete minicell phenotype (6), making the existence of *minA* doubtful. The *minB* locus is defined by the classical, and until now the only, well-characterized *E. coli* minicell mutant allele (henceforth called *minB1*) of Adler et al. (1) and is located at 25.6 map units on the *E. coli* chromosome (6, 23). *minB* is thus not part of other known cell division gene clusters (for reviews, see references 7, 11, and 12).

In this paper we report the isolation of the *minB* locus from *E. coli* and demonstrate that both overexpression and underexpression of this locus result in the minicell phenotype. The isolated locus codes for several gene products that are associated with *minB* function and are therefore likely to play a role in the localization of the division septum.

MATERIALS AND METHODS

General genetic and recombinant DNA techniques. P1 transduction, DNA-transformation, λ infection, selection for λ lysogens, and in vivo recombination between plasmid and phage DNA were performed as previously described (18, 24). Other procedures were performed essentially as described by Maniatis et al. (17).

Media, strains, plasmids, and phages. Cells were grown either in L broth or on L plates. When relevant, antibiotics were added to 50 μg/ml (ampicillin, kanamycin sulfate) or 25 μg/ml (tetracycline).

Strains, plasmids, and bacteriophages are listed in Table 1. Strains PB103 and PB104 were obtained by P1-mediated transduction of JK268 to *purB*$^+$ from strain χ1081. Strain PB111 was constructed by P1-mediated transduction of N6377 to tetracycline resistance from ED18.

Strains PB103T_{mk}3, 17, 32, and 63 and N6377T_{mk}17 were obtained by inoculation of 100 μl of an overnight culture of strain PB103 or N6377 with 2×10^5 PFU of λDB37T_{mk}3, 17, 32, or 63 (*imm*^{21}) in 100 μl of phage buffer. (T_{mk} indicates a mini-Kan transposon, element 9 in plasmid pNK862 [27].) After 20 min at room temperature 0.5 ml of L broth was added; the mixture was incubated at 37°C (or 30°C for N6377) for 1 h and then plated out in soft agar on an L plate containing kanamycin. Kanamycin-resistant (Kan^r) colonies were screened for lysogeny by cross-streaking against λB10 (*imm*^{21}) and λL47 (*imm*^{434}). In all cases a surprisingly high percentage of Kan^r colonies (50 to 70%) were nonlysogenic for λ (λB10^s λL47^s) and had become MinB$^-$ (see below). The presence of T_{mk} and absence of λ sequences in these strains was confirmed by Southern blot and hybridization analyses.

Plasmid pDB100 was obtained by subcloning the 5.0-kilobase-pair (kbp) *Eco*RI-*Pvu*II chromosomal fragment from λDB37 into *Sal*I-*Pvu*II-digested λSV2 (13, 14) via a pUC13-derived intermediate subclone (pDB91; data not shown) carrying the 9.2-kbp *Eco*RI fragment of λDB37 inserted into the unique *Eco*RI site of pUC13. Derivatives of

* Corresponding author.

TABLE 1. Strains, plasmids, and phages

Strain, plasmid, or phage	Relevant markers	Source
E. coli		
JK268	*dadR trpE trpA tna purB*	B. Bachmann
χ1081	*lacY proC* T6^r, *minB1 purE his* Str^r T3^r *xyl ilv* CycA^r CycB^r *met*	R. Curtiss III
ED18	χ1081 *zcf-117*::Tn*10*	This laboratory
ED28	χ1081 *srl*::Tn*10 recA*	This laboratory
N4956	C600 r_k^- m_k^+	NIH[a] collection
N6377	N4956 (λ *cI857 bio*936ΔS-XΔH1)	B. Howard
N100	*pro recA*	NIH collection
PB103	JK268 *purB*$^+$	This work
PB104	JK268 *purB*$^+$ *minB1*	Ths work
PB103T_{mk}3	PB103 *minB*::T_{mk}3	This work
PB103T_{mk}17	PB103 *minB*::T_{mk}17	This work
PB103T_{mk}32	PB103 *minB*::T_{mk}32	This work
PB103T_{mk}63	PB103 *minB*::T_{mk}63	This work
PB111	N6377 *zcf-117*::Tn*10 minB1*	This work
N6377T_{mk}17	N6377 *minB*::T_{mk}17	This work
Plasmids[b]		
pSE110 (pSC101)	*umuDC*$^+$ Kan^r	G. Walker
pNK862 (pBR322)	*ptac*$^+$ T_{mk} Kan^r Amp^r	N. Kleckner
λSV2 (λ)	λ *att*$^+$ Amp^r Cam^r	B. Howard
pDB100 (λ)	λSV2 *minB*$^+$ Cam^s	This work
pDB100T_{mk}3 (λ)	pDB100 *minB*::T_{mk}3	This work
pDB100T_{mk}17 (λ)	pDB100 *minB*::T_{mk}17	This work
pDB100T_{mk}32 (λ)	pDB100 *minB*::T_{mk}32	This work
pDB100T_{mk}63 (λ)	pDB100 *minB*::T_{mk}63	This work
pFA-mp11 (F)	Amp^r	S. Bourgeois
pDB101 (F)	pFA-mp11 *minB*$^+$	This work
pMLB1107 (pBR322)	*laqI*^{q} *lacZ*$^+$ Amp^r	M. Berman
pDB102 (pBR322)	pMLB1107 *laqI lacZ minB*$^+$ Amp^r	This work
pDB102T_{mk}17 (pBR322)	pDB102 *minB*::T_{mk}17	This work
pDB103 (pBR322)	pMLB1107 *lacZ* P_{lac}::*minB* Amp^r	This work
Phages		
λB10	*imm*^{21} *vir*	NIH collection
λL47	*imm*^{434} *vir*	NIH collection
λW30	*imm*λ *vir*	NIH collection
λR5	*imm*λ R$^-$ (amber)	NIH collection
λD69	λBam1^0 *srl*(1-2)$^\Delta$ *att*$^+$ *imm*^{21} *nin5 shn6*^{0}	NIH collection
λDB37	λD69 *int minB*$^+$	This work
λDB37T_{mk}3	λDB37 *minB*::T_{mk}3	This work
λDB37T_{mk}17	λDB37 *minB*::T_{mk}17	This work
λDB37T_{mk}32	λDB37 *minB*::T_{mk}32	This work
λDB37T_{mk}63	λDB37 *minB*::T_{mk}63	This work
λNT5	*imm*^{21} *lacZ' bla'*	N. Trun
λDB103	λNT5, *laqI*^{q}, P_{lac}::*minB* Amp^r	This work

[a] NIH, National Institutes of Health.
[b] Plasmid origins are indicated within parentheses.

pDB100 carrying each of the four *minB*::T_{mk} alleles in place of *minB*$^+$ were obtained by in vivo recombination between the pDB100 plasmid and the respective chromosomal *minB*::T_{mk}alleles. To this end pDB100 was first transformed into the haploid PB103T_{mk} strains described above. Approximately 300 ng of plasmid DNA from each of the transformants was then used to transform strain N100 to ampicillin resistance (Amp^r) and Kan^r. In each case one to six transformants were obtained. In all cases these harbored the desired *minB*::T_{mk} plasmids as confirmed by restriction enzyme analysis of plasmid DNA.

Plasmid pDB101 was obtained by inserting a 5.8-kbp *Eco*RI-*Bam*HI fragment from pDB100 (including the 5.0-kbp chromosomal *Eco*RI-*Pvu*II *minB*$^+$ fragment) into the multiple cloning site of the mini-F vector pFA-mp11 (A. Koop, M. Hartley, and S. Bourgeois, personal communication).

Plasmid pDB102 was prepared by ligating the same fragment to the 6.3-kbp *Eco*RI-*Bam*HI fragment of the pBR322 derivative pMLB1107. pMLB1107 contains the mp8 multiple cloning site flanked by the *lac* regulatory region and by *lacZ* sequences (M. L. Berman and S. Crush-Stanton, personal communication). Plasmid pDB102 lacks the 1.3-kbp *EcoRI* fragment of pMLB1107 that contains the *laq*I^q gene and the *lac* operator and promotor region. Derivatives of pDB102 carrying T_{mk} were obtained by in vivo recombination as described above for pDB100.

Plasmid pDB103 was obtained by cloning a 2.7-kbp fragment from a partial *Sau*3AI digest of pDB100 into the unique *Bam*HI site of pMLB1107, thereby placing part of the *minB* locus under control of the regulatory region of the *lac* operon. pDB103 was identified on the basis of its ability to induce the minicell phenotype in wild-type cells in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG).

Recombinant phage λDB37 (*minB*$^+$) was obtained as described below. λDB103 was obtained by in vivo recombination between pDB103 and λNT5. λNT5 contains the 3′ parts

of the *bla* and *lacZ* genes organized to permit homologous recombination to occur between this phage and plasmids containing cloned DNA inserted between *bla* and *lacZ* sequences (N. Trun, personal communication).

Determination of MinB phenotypes. For rapid screening a colony was picked with a toothpick from an overnight plate, suspended in a drop of saline on a microscope slide, and examined by phase-contrast microscopy. The phenotypes were confirmed by growing cells in liquid medium to an optical density at 600 nm of 0.6 to 0.7, followed by fixation in 0.5% glutaraldehyde for 15 min at room temperature. Cells were then spun down at 13,000 × *g* for 3 min, washed once in saline, and suspended in 1/10 the original volume of saline. A small sample (2 to 3 μl) was applied to a microscope slide previously coated with polylysine by dipping the slide in a solution of 2 mg of polylysine per ml of water and drying in air. Strains were defined as MinB$^-$ when all three of the following were present: cells with polar septa, large numbers of small spherical minicells, and substantial numbers of filamentous cells of various lengths.

Selection of nonlysogenic segregants. Selection for nonlysogenic segregants from a PB104(λDB37) lysogen was performed by a procedure kindly made available by A. Das (personal communication). The procedure is based on the observation that λR5 (*imm*λ endolysin negative), which is itself unable to kill cells, will upon heteroimmune infection transactivate lysogenic phages of type imm^{21} (such as λDB37). Cells (10^3) of the PB104(λDB37) lysogen were mixed with 10^9 PFU of λR5 on an EMBO plate (10) on which lysogens form intensely red colonies and nonlysogens form less intensely colored ("white") colonies. After overnight growth, 16 colonies that appeared white were cross-streaked against λB10 (imm^{21}) and λW30 (*imm*λ). Four proved to be true nonlysogens. Two of these had retained the wild-type phenotype of the lysogen, whereas two had become MinB$^-$ (see the text).

Transposon mutagenesis of λDB37. Phage λDB37 was mutagenized with T_{mk}. A plate lysate of λDB37 was prepared by growing the phage on a strain carrying pNK862 in the presence of 2.8 mM IPTG. A 0.1-ml sample (2×10^6 PFU) of the lysate was added to 0.1 ml of an overnight culture of strain PB104. After incubation at room temperature for 15 min, 1.0 ml of L broth was added, and the incubation was continued for 1 h at 37°C. L soft agar was then added, and the mixture plated on an L plate containing kanamycin to select for Kan^r lysogens. Colonies were tested for lysogeny by cross-streaking against λB10 and λL47. Of 63 true lysogens (Kan^r λB10^r λL47^s), four had retained the MinB$^-$ phenotype of PB104 and thus presumably contained derivatives of λDB37 that carried *minB* alleles interrupted by T_{mk}. The sites of transposon insertion in the λDB37T_{mk} phages were initially determined by Southern analysis of genomic DNA from strains lysogenic for the phages and later confirmed by endonuclease restriction analysis of plasmids carrying the same *minB*::T_{mk} alleles.

Maintenance of λSV2 and derivatives. To maintain λSV2 or its derivatives as a single-copy plasmid, the plasmid was transformed into strains lysogenic for the defective prophage λ *c*I857 *bio*936Δs-XΔH1 as described previously (14). To maintain the constructs as autonomously replicating multicopy plasmids, they were transformed to strains nonlysogenic for λ.

Identification of proteins. Labeling of plasmid-encoded polypeptides in maxicells was performed as described previously (3), except that the strain used was N100 and labeling took place for 30 min. In the case of plasmid pDB103, 1 h before the addition of [^{35}S]methionine the maxicell suspension was split into two equal portions and IPTG (9 mM) was added to one of the portions.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed essentially as described previously (15). The gels contained 13% acrylamide–0.7% bisacrylamide. After electrophoresis the gels were treated with En^{3}Hance (Du Pont Co.), dried, and placed on Kodak XAR-5 film.

RESULTS

Cloning of *minB*. There is as yet no direct method to discriminate between the MinB$^+$ and MinB$^-$ phenotypes other than microscopic examination of cells. Therefore, for isolation of the *minB* locus we made use of the close proximity of *minB* to the *umuDC* operon (linked >90% by P1 transduction [unpublished data]) by screening recombinant clones containing *umuDC* sequences for their ability to correct minicelling in a *minB1* mutant strain.

Random genomic *Sau*3AI fragments (9 to 12 kbp) from strain PB103 (*minB*$^+$) were inserted into the unique *Bam*HI site of λD69 (19). The resulting library was screened by plaque hybridization with a 4.3-kbp *Hin*dIII fragment of plasmid pSE110 containing cloned *umuDC* sequences (8). Hybridizing phages were purified and used to lysogenize strain PB104 (*minB1*). Lysogens were then examined by phase-contrast microscopy to identify recombinant phages that had corrected the minicell phenotype of the host.

One phage, λDB37, completely corrected the minicell phenotype of PB104 and was chosen for further study. A physical map of the chromosomal insert in λDB37 (Fig. 1a) showed that λDB37 contained a 9.5-kbp chromosomal insert, including part of *umuC* and the whole of *umuD*, extending from *umuD* toward *purB* (8).

Derivatives of λD69 that carry an insert in the *Bam*HI site are *int* mutants (19). Therefore, if λDB37 carries *minB*$^+$, lysogeny should occur primarily by a single crossover event in the *minB-umuCD* region of the chromosome. Consequently, phages released from a PB104(λDB37) lysogen should carry either *minB*$^+$ or *minB1*, leaving a single copy of the other allele in the host chromosome. To verify this, two experiments were performed.

First, spontaneously released phages in the supernatant of a PB104(λDB37) culture were tested for their ability to correct the *minB1* phenotype upon relysogenization of strain PB104. Of 12 phages, 4 were unable to correct the minicell phenotype and thus presumably had obtained the original *minB1* allele of the host. Second, four nonlysogenic segregants from the same PB104(λDB37) culture were also examined. Two of these retained the wild-type phenotype of the lysogen despite loss of the λ prophage and thus presumably had obtained the *minB*$^+$ allele of λDB37. These results confirmed that the bacteriophage genome contained sequences that flanked the site of the *minB1* mutation and thus that λDB37 contained all or part of the *minB*$^+$ gene.

The sequences required for complementation of the *minB1* mutation were further localized by subcloning a 5.0-kbp *Eco*RI-*Pvu*II chromosomal *minB*$^+$ fragment from λDB37 into the plasmid vector λSV2, giving rise to pDB100 (Fig. 1b). Because λSV2 contains the λ *att* site, the plasmid and its derivatives can be maintained as a single copy integrated at the chromosomal *att*λ site when introduced into strains lysogenic for the defective prophage λ *c*I857 *bio*936ΔS-XΔH1. Alternatively, the plasmids can be maintained as multiple-copy plasmids that use the lambda origin of replication by introducing it into strains lacking a λ prophage (13, 14).

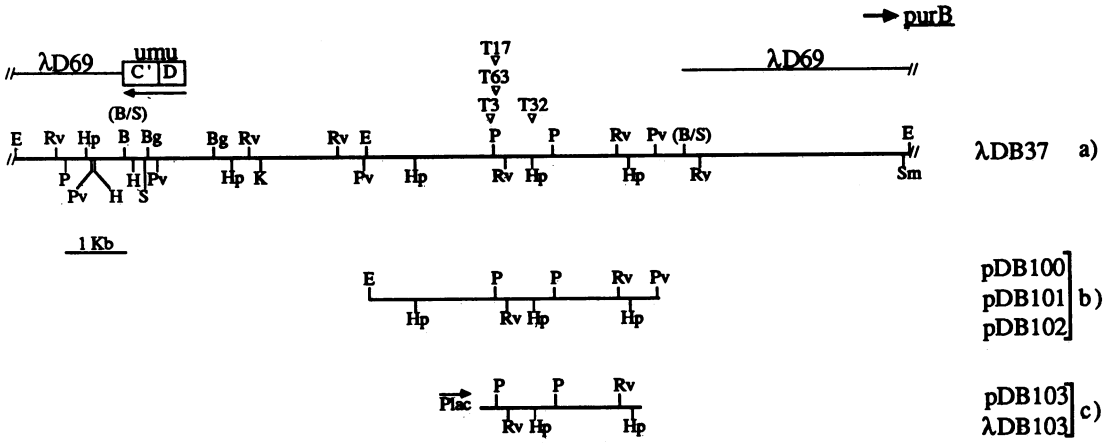

FIG. 1. Physical map of the cloned *E. coli minB* region. (a) Chromosomal insert of λDB37. (b) Chromosomal insert in plasmids pDB100, pDB101, and pDB102. (c) Chromosomal fragment present in pDB103 and λDB103. The position of the *lac* promotor and the direction of transcription are indicated. Also indicated are λD69 flanking sequences in λDB37, the position and direction of transcription of the *umuDC* operon, the orientation of the *minB* fragment relative to *purB*, and the sites of transposon insertions (▽) which abolished *minB* functions. Restriction sites: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*II; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; Rv, *Eco*RV; S, *Sal*I; Sm, *Sma*I. B/S, *Bam*HI-*Sau*3AI junction.

Integration of pDB100 into the chromosomal *att*λ site of strain PB111 corrected the *minB1* phenotype of the host, confirming the presence of *minB*$^+$ on the 5.0-kbp fragment (Table 2; see Fig. 3b). The same chromosomal fragment also complemented the *minB1* allele in strain ED28 (Table 2) when cloned into a mini-F vector (pDB101). Thus, when present in single copy *minB*$^+$ is dominant over *minB1* in *trans*, implying that the *minB1* mutation results in loss of a functional *minB* gene product.

TABLE 2. Minicell phenotypes of cells containing the cloned *minB* locus[a]

Plasmid or bacteriophage	Expt no.	Min phenotype on host strain:	
		minB1	*minB*$^+$
Single copy integrated			
λSV2	1	−	+
pDB100	2	+	+
pDB100T_{mk}[b]	3	−	+
λDB103 (−IPTG)	4	−	+
λDB103 (+IPTG)[c]	5	+	+
Mini-F pDB101	6	+	+
Multiple copies			
λSV2	7	−	+
pDB100	8	−	−
pDB100T_{mk}[d]	9	−	+
pMLB1107	10	−	+
pDB102	11	−	−
pDB102T_{mk}17	12	−	+
pDB103 (−IPTG)	13	±	+
pDB103 (+IPTG)[c]	14	−	−

[a] The indicated plasmids and bacteriophages were introduced into *minB*$^+$ or *minB1* cells, and the resulting strains were grown and examined for phenotype (−, minicell phenotype; +, wild-type phenotype). Most plasmid constructs were tested in several *minB1* and *minB*$^+$ strains of different background. The *minB1* strains used were PB111 (experiments 1 through 3), PB104 (experiments 4 through 14), and ED28 (experiment 6). Wild-type strains used were N6377 (experiments 1 through 3), PB103 (experiments 4 through 14), N100 (experiments 6 through 14), and N4956 (experiments 7 through 9).

[b] T_{mk}, T_{mk}3, 17, 32, and 63.

[c] IPTG was added to 0.5 mM; concentrations of 0.1 and 0.05 mM gave rise to intermediate phenotypes (small number of minicells and occasional short filaments).

[d] T_{mk}, T_{mk}3, 17, and 63. The effect of pDB100T_{mk}32 on cell phenotypes could not be determined because the plasmid became highly unstable upon autonomous replication for an as yet unknown reason.

Transposon mutagenesis of *minB*$^+$. After mutagenesis of phage λDB37 with a mini-Kan transposon (T_{mk}), four independent T_{mk} derivatives were isolated that were unable to correct the minicell phenotype of PB104 upon lysogenization (λDB37T_{mk}3, 17, 32, and 63). The transposon insertions are therefore assumed to lie within the functional *minB*$^+$ region of λDB37. The sites of the insertions indicate that *minB* is located on the *E. coli* chromosome 5 to 6 kbp counterclockwise from the *umuDC* operon (Fig. 1a) (rather than clockwise [2]).

The chromosomal *minB*$^+$ alleles of strains PB103 and N6377 were replaced by each of the four *minB*::T_{mk} alleles by an in vivo recombination technique (see Materials and Methods). The *minB*::T_{mk} strains thus obtained were viable but in all four cases exhibited the classical MinB$^-$ phenotype (Fig. 2). These results support the view that a functional

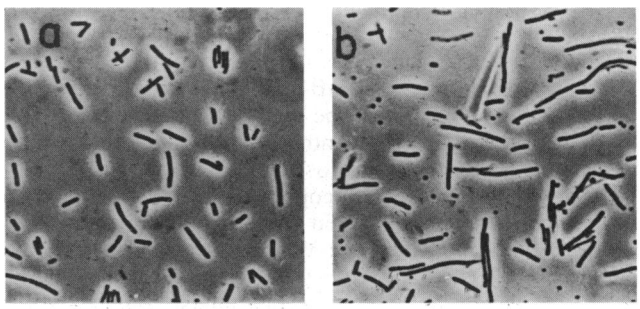

FIG. 2. Phase micrographs showing the minicell phenotype caused by insertional mutagenesis of the chromosomal *minB* allele. (a) N6377(λSV2). (b) N6377T_{mk}17(λSV2). Both strains were lysogenic for λSV2. Cells were grown in LB plus ampicillin. Strains containing *minB*::T_{mk}3, 32, and 63 (not shown) appeared identical to N6377T_{mk}17.

minB locus is necessary to retain the normal division pattern.

Multiple copies of *minB*$^+$ induce the minicell phenotype in wild-type cells. As noted above, when integrated as a single copy at the *att*λ site of strain PB111, pDB100 corrected the minicell phenotype of the host. Surprisingly, however, pDB100 failed to correct the *minB1* mutant phenotype when permitted to replicate as a multicopy plasmid in *minB1* hosts (Table 2). The possibility that the absence of *minB1*-complementing activity reflected loss of *minB* sequences from pDB100 during autonomous replication was excluded by isolating plasmid DNA from a minicelling PB104(pDB100) transformant and reintroducing it into the *att*λ site of strain PB111. Of the 65 PB111(pDB100) integrants tested, all had obtained the wild-type phenotype.

The unexpected failure of the multicopy *minB*$^+$ plasmid to correct the minicell phenotype of *minB* mutants was subsequently explained by the observation that pDB100 induced the minicell phenotype when introduced into wild-type strains (Table 2, Fig. 3g). The phenotype induced by this plasmid in several *minB*$^+$ hosts resembled that of the classical *minB1* mutant (Fig. 3a). Similar results were obtained with pDB102 (Table 2), in which the 5-kbp chromosomal insert of pDB100 was present in the multicopy plasmid vector pMLB1107. These results showed that multiple copies of the chromosomal insert induce the minicell phenotype.

Evidence that correction of minicell formation in *minB1* cells and induction of minicell formation in *minB*$^+$ cells were both a function of the *minB* genetic unit came from studies of the *minB*::T_{mk} transposon insertions described above. When the four *minB*::T_{mk} alleles were crossed into pDB100, the resulting T_{mk} derivatives (pDB100T_{mk}3, 17, 32, and 63) were unable to correct minicell formation in strain PB111 when present as a single copy at the *att*λ site (Table 2, Fig. 3c) and also failed to induce minicell formation in wild-type strains when allowed to replicate in the multicopy mode (Table 2, Fig. 3h). Thus, induction of minicell formation was caused by the presence of multiple copies of *minB*$^+$, suggesting that overexpression of this locus causes the same phenotype as underexpression.

Both underexpression and overexpression of the *minB* locus cause the minicell phenotype. Confirmation that both underexpression and overexpression of *minB* can lead to minicell formation came from studies of a P_{lac}::*minB* transcriptional fusion in which part of the *minB* locus was placed under control of the IPTG-inducible *lac* promotor (Fig. 1c).

When a single copy of P_{lac}::*minB* (on λDB103) was integrated at the chromosomal *att*λ site of PB104, correction of the mutant phenotype of the host required growth in the presence of IPTG (Table 2, Fig. 3d and e). This confirmed that the fusion placed MinB$^+$ (defined as the ability to correct the mutant phenotype of the classical *minB1* mutation) under the obligatory control of P_{lac} and implied that the *minB1* mutation resulted in loss of *minB* expression.

When present on the multicopy plasmid pDB103, the same P_{lac}::*minB* fusion only induced the full minicell phenotype in wild-type strains when the cultures were grown in the presence of IPTG (Table 2, Fig. 3i and j). When present in a *minB1* strain the multicopy plasmid almost completely corrected the *minB1* phenotype in the absence of IPTG (Table 2). This presumably reflected the basal level of uninduced transcription from the multiple copies of P_{lac}::*minB* present in the cells. Upon addition of IPTG the full minicell phenotype reappeared.

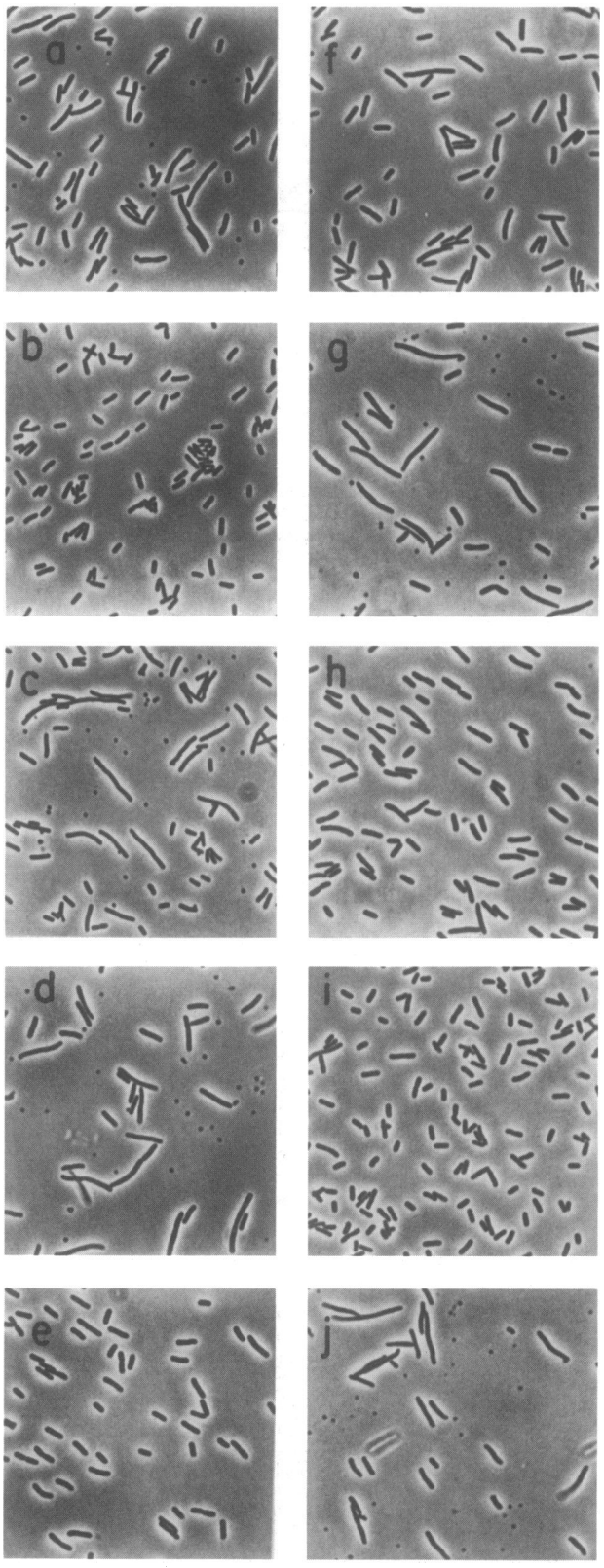

FIG. 3. Phase micrographs showing correction and induction of the minicell phenotype by the cloned *minB* locus. The indicated bacteriophages or plasmids were present either as lysogens (a through e) or as multicopy plasmids (f through j). (a) PB111(λSV2); (b) PB111(pDB100); (c) PB111(pDB100T_{mk}17); (d) PB104(λDB103) grown without IPTG; (e) PB104(λDB103) grown in the presence of 1 mM IPTG; (f) N4956(λSV2); (g) N4956(pDB100); (h) N4956 (pDB100T_{mk}17); (i) PB103(pDB103) grown without IPTG; (j) PB103(pDB103) grown in the presence of 3 mM IPTG. Strains were grown in LB plus ampicillin.

Thus expression of the portion of the *minB* locus that is present in the fusion must be maintained within certain levels to prevent the minicell phenotype.

Polypeptides encoded by the *minB* region. To identify polypeptides encoded by the *minB* region, maxicell experiments were performed on plasmid pDB102. Five peptide bands (bands 1 through 5 in Fig. 4) were identified whose synthesis was directed by the chromosomal insert of pDB102 (Fig. 4, lanes c and e). The apparent molecular weights of the peptides were as follows: 1, 30,000; 2, 25,000; 3, 19,500; 4, 16,000; and 5, 15,500. Evidence that all five peptides were associated with the functional *minB* genetic unit came from the demonstration that synthesis of all five peptides was markedly affected by the T_{mk} 17 insertion (lane d) that abolishes both the complementing and minicell-inducing activities of the complete locus (Fig. 3, Table 2). It is not yet known whether all five peptides represent primary translation products.

Plasmid pDB103 did not direct the synthesis of peptide 2 (Fig. 4, lanes f and g), suggesting that part of the coding region for this protein lies to the left of the chromosomal insert present in pDB103. Since pDB103 is able to both correct and induce the minicell phenotype, we conclude that the band 2 protein is not required for these functions of the *minB* locus. In addition, in cells containing pDB103, the band 4 peptide was replaced by a smaller peptide with an apparent molecular weight of 15,000 (Fig. 4, lanes f and g). The reason for this has not been established.

Synthesis of the other *minB*-directed peptides from pDB103 appeared to be enhanced in the presence of IPTG (Fig. 4, lanes f and g), consistent with the fact that the *minB* functions of pDB103 are IPTG inducible. It should be noted that since IPTG was added to cells that had been subjected to a lethal dose of UV radiation, the level of induction of *minB* products seen here did not necessarily reflect the in vivo situation.

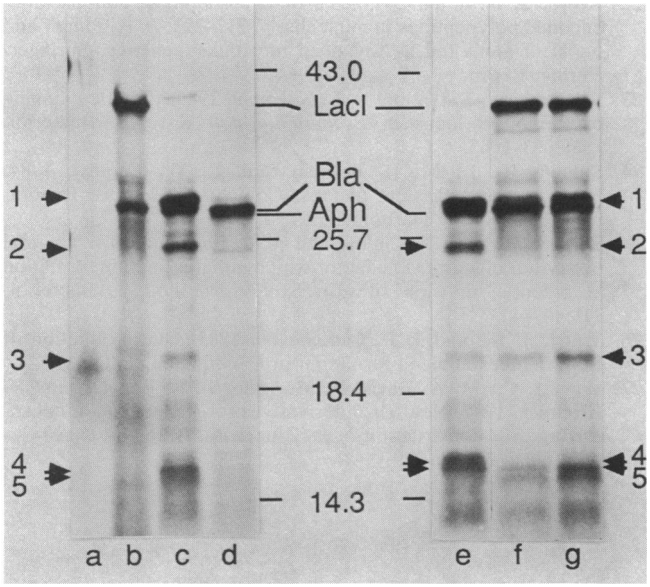

FIG. 4. Identification of polypeptides encoded by the *minB* region. Maxicell experiments were performed on cells containing the following plasmids (lanes): a, no plasmid; b, pMLB1107; c, pDB102; d, pDB102T_{mk}17; e, pDB102; f, pDB103; g, pDB103 plus IPTG. Indicated are the positions of lactose repressor (LacI) and β-lactamase (Bla); the kanamycin resistance determinant aminoglycoside 3′-phosphotransferase (Aph; see reference 21) encoded by T_{mk}, which migrates slightly faster than Bla; and *minB* peptides 1 through 5 (see text). Molecular size markers are indicated in kilodaltons.

The observation that *minB* expression in λDB103 and pDB103 was IPTG dependent indicates that the natural *minB* promoter lies outside the chromosomal fragment present in these constructs. Taken together, the above results make it likely that the *minB* locus is an operon in which the gene for peptide 2 lies promoter proximal to the sequences required for the minicell-inducing and *minB1*-complementing activities of the locus.

DISCUSSION

In this report we describe the isolation and initial characterization of the *minB* locus. The fact that loss of *minB* functions resulting from transposon mutagenesis was accompanied by the disappearance or marked decrease in synthesis of several peptides whose synthesis was directed by the cloned *minB* region indicates that the functional *minB* genetic unit is a complex locus coding for several gene products.

The most striking finding of the present study was the observation that both overexpression and underexpression of the locus interfered with the normal placement of the division septum. This was most clearly shown by the appearance of minicells and of filaments of intermediate lengths when *minB* expression was diminished by the presence of transposon insertions or when expression was increased by induction of an exogenous promoter. Therefore, the normal cell division process requires that the *minB* gene product(s) be maintained within certain levels.

It is not known whether the overproduction and underproduction phenotypes reflect different concentration-dependent properties of a single gene product. One could imagine, for example, the concentration-dependent polymerization of a *minB* gene product resulting in formation of an inactive protein that competes with the active monomeric species. Alternatively, since *minB* is a complex locus it is possible that different gene products are involved in the overproduction and underproduction phenotypes.

The finding that underexpression and overexpression of a genetic locus give rise to a similar phenotype is rare but not without precedent. Recently it was reported that an elevated gene dosage of the *Saccharomyces cerevisiae* transcription factor SPT6 caused a mutant phenotype similar to that caused by a reduced gene dosage (4). The authors raised the possibility that such a phenomenon occurs when the gene product under study is part of a multicomponent structure in which a strict stoichiometry between the components must be maintained for proper functioning of the complex. Underproduction and overproduction of one of the components would therefore lead to a nonfunctional complex. In this regard it will be of interest to investigate whether the *minB* gene product(s) interacts with the product of the essential cell division gene *ftsZ*, since it was reported recently that overexpression of *ftsZ* also induces minicell formation in wild-type cells (26).

The roles of the *minB* gene products in the cell division process remain to be defined. The finding that polar transposon insertions in the chromosomal *minB* allele are not lethal suggests that the locus is not essential for the survival of the cell under laboratory conditions. A true *minB* deletion mutant will be needed to prove this point.

Several years ago Teather et al. (25) proposed that *minB* may be required to inactivate residual division sites at the cell poles. In this view, in the absence of the *minB* product

the polar site can function again as the site of formation of a new septum, the minicell septum. A possible candidate for such a polar site is the polar periseptal annulus that remains from the preceding division event (5, 16). Further study of the minicell locus should provide information regarding the validity of this provocative model.

The isolation of this locus may also be helpful to investigators who wish to use minicells of bacteria other than *E. coli*, since we found that IPTG induction of the P_{lac}::*minB* fusion also induced the minicell phenotype in *Salmonella typhimurium* LT2 (B. C. M. McGrath and P. A. J. de Boer, unpublished results).

Finally, the findings in this report emphasize the desirability of using single-copy vectors during the initial stages of cloning biologically active genes. The fact that cloned *minB*$^+$, when present in multiple copies per cell, did not correct the mutant phenotype complicated previous attempts, in this laboratory and elsewhere, to isolate this locus.

ACKNOWLEDGMENTS

We thank M. Berman, S. Bourgeois, A. Das, B. Howard, N. Trun, G. Walker, and S. Weller for their gifts of strains, plasmids and phages. In addition, we thank A. Das for many helpful suggestions.

This work was supported by National Science Foundation grant 8500314 and Public Health Service grant A122183 from the National Institutes of Health.

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